# **Production of Lactic Acid From Alfalfa Fibrous Fraction**

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#### Introduction

Fractionation of alfalfa herbage is carried out to yield a number of value-added products including enzymes (from transgenic plants), carotenoids, and soluble and particulate protein concentrates. The value of these products can be in the range of \$1000 - \$2000 per acre-year. The fibrous component from fractionation is about 75% of the initial herbage dry matter. While it makes a good feed for ruminants, other economic uses are being evaluated. Since a large part of the fiber is made up of polymerized sugars, it is possible to hydrolyze the fiber into sugars, by means of selected enzymes, and then to ferment it into target chemicals using appropriate microorganisms.

Both ethanol and lactic acid have been produced through fermentation of saccharified alfalfa. Lactic acid appeared more promising because of its higher yield and higher unit price. Cargill has developed a process for making a biodegradable plastic ("ECO-PLA") from lactic acid. This could create a large market for lactic acid.

### **Methods**

Alfalfa fibrous fraction, resulting from wet fractionation was fermented with or without a liquid hot water (LHW) pretreatment (220°C for 2 min.). The pretreatment partially hydrolyzes the material resulting in a liquid and solid fraction referred to as "extract" and "residue".

The untreated fiber and the extract and residue from the pretreatment respectively were dosed with commercially available enzymes found to be effective for saccharification in previous work and were inoculated with one of four species of *Lactobacillus*. The four species of *Lactobacillus* were: *delbrueckii*, *plantarum*, *pentoaceticus*, and *xylosus*. Fermentations were carried out at near-optimum conditions for the respective species. Levels of lactic acid, acetic acid, and unfermented sugars were monitored periodically.

### **Results**

The enzymatic hydrolysis of plant fiber to sugars results in both six-carbon sugars (hexoses) and fivecarbon sugars (pentoses). Most organisms found in nature can ferment one or the other but not both. L. plantarum is an exception with the ability to ferment both hexoses and pentoses. Since the extract resulting from the LHW pretreatment is rich in pentoses and the residue is rich in hexoses, the ability of L. plantarum to ferment pentoses is shown by comparing yields for the four organisms from the extract (Fig. 1). Figure 2 shows roughly equal production for the four species from the hexose-rich residue. Figure 3 shows total yields from the extract plus the residue. Here the yields from L. plantarum after LHW pretreatment are roughly 44% greater than for L. delbrueckii because of the ability of the former to ferment pentoses. Figure 4 shows relative lactic acid yields by L. plantarum

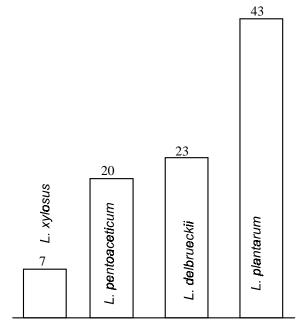


Figure 1. Lactic acid yield from LHW extract resulting from 100g alfalfa fiber.

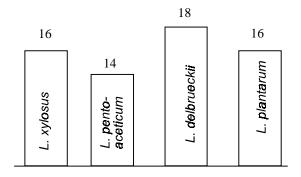


Figure 2. Lactic acid yield from LHW residue resulting from 100g alfalfa fiber.

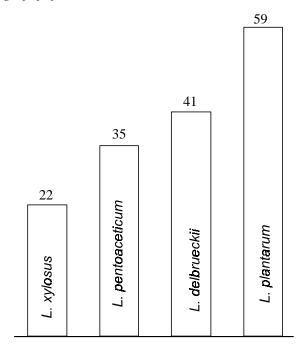


Figure 3. Total lactic acid yield from 100g alfalfa fiber subjected to LHW pretreatment.

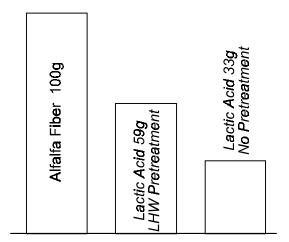


Figure 4. Lactic acid yields from 100g alfalfa fiber using L. plantarum with and without LHW pretreatment.

with and without the LHW pretreatment. While the LHW pretreatment lead to an almost 80% yield increase in this case, the economic advantage of the pretreatment is still unclear because of its considerable capital and operating cost.

The 33g of lactic acid production for the untreated fiber shown in Fig. 4 occurred at 24 h of fermentation. At this time there was 15g of unfermented sugars and 5g of acetic acid for a total of 53g of hydrolysis products. It appears therefore, that, with improved management, there may be the potential for increasing the yield of lactic acid from untreated fiber.

## **Conclusions**

Yields as high as 59g of lactic acid were obtained from 100g of pretreated alfalfa fiber using *L. plantarum*. While yields from untreated fiber were only 56% of this, there appears to be potential for increasing this yield.